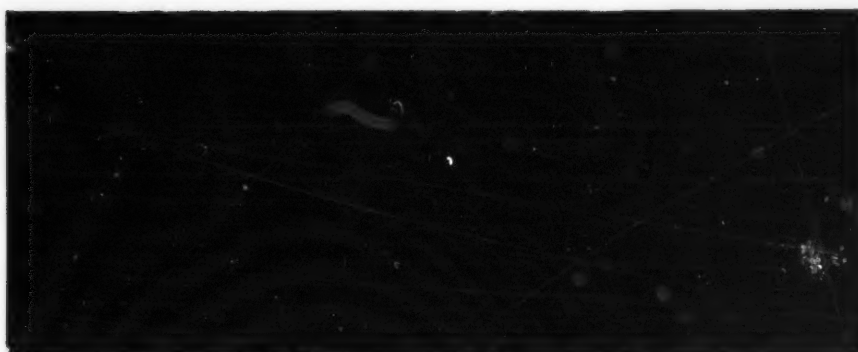




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ANTINUTRITIVE CONSTITUENTS IN KOCHIA:

PHENOLIC ACIDS, PHENOLS, FLAVONOLS, SAPONINS AND TANNINS

EFFECTS OF STAGE OF
MATURITY AND LOCATION

by

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EXECUTIVE SUMMARY

The levels of antinutritive constituents, phenols, phenolic acids, flavonols, tannins and saponins, and content and composition of fibre were determined in several samples of kochia.

The following samples were analyzed:

- good quality (lush, green vegetation, tall plants) and poor quality (yellow, short plants) kochia harvested from the wild stand in Saskatoon, at five stages of maturity.
- kochia grown on saline site at Swift Current, harvested at five stages of maturity.
- kochia grown in Saskatchewan from seeds native to the province and from seeds grown in Texas.
- kochia samples from New Mexico.
- kochia plants with red stems - red flowers and green stems - yellow flowers.

Fibre content was influenced by the quality of kochia forage. Poor quality, yellow plants contained more fibre than good quality green kochia harvested from the same location. Poor quality kochia contained more of all antinutritive components as compared to good quality sample.

There was a location/environmental difference among samples of good quality kochia grown in Saskatchewan. More of the antinutritive constituents accumulated in forage grown on saline than on regular soil. All antinutritive components accumulated mainly in leaves.

The relatively high amounts of phenolic acids and flavonols in kochia, at the later stages of maturity, suggest that these compounds could be contributing to the growth inhibiting effect of kochia plant debris on the next year's plant growth.

The highest concentrations of the four antinutritive components were found in kochia from New Mexico. Texas-origin kochia contained less of these components than Saskatchewan-origin kochia.

Small differences in antinutritive constituent contents were determined between kochia with red stems and red flowers and those with green stems and yellow flowers.

1. INTRODUCTION

Phenolic constituents of plants are known to be responsible for colour, astringency and bitterness of forage. Certain phenolic acids, e.g. p-coumaric and ferulic, are apparently ester-linked at their carboxyl groups to cell wall polysaccharides and are considered to play an important role in degradation of plant organic matter in soil (Hartley and Haverkam, 1984). They are also reported to influence the microflora of rumen and slow down the digestion process (Chesson et al. 1982).

Lignins are polyphenolic compounds determined in plant materials as a constituent of fibre. Their precursors are p-coumaric, ferulic and sinapic acids (Akin, 1982). Rumen microorganisms are able to hydrolyse lignin, but the released phenolic compounds have antimicrobial properties against bacteria and fungi. Therefore their presence in rumen will interfere with the further digestion of forage.

Another polyphenolic constituent, tannin, consists of catechins with some content of gallic and ellagic acids. Tannins provide natural protective system against birds, however, their bitterness is a deterrent in animal feeding. It has been reported that tannin rich diets decrease growth of experimental animals (Price et al. 1979).

In addition to phenolics there are also saponins which, through their bitterness, decrease palatability of animal diet. Crude kochia saponin contains the sapogenin oleanolic acid (Kernan et al. 1973). Saponins were believed to be the cause of kochia toxicity to cattle in Argentina (Sonto and Milano 1966)

High fibre content, although fibre is not considered to be an antinutrient, decreases digestible matter of plant and also interferes with digestion of forage.

Phenolic acids such as ferulic and p-coumaric were found in kochia forage (Lodhi, 1979) and were believed to be factors in the autoallelopathy of kochia, i.e., chemicals released by first year plant debris which inhibited the growth of second year plants. Flavonols, e.g., quercetin and myricetin, were also isolated from kochia and shown to be toxic to young kochia seedlings (Lodhi, 1979).

Therefore, the objectives of this study were as follows:

1. to determine the level of these antinutritive constituents in good and poor quality kochia grown at the same location,
2. to determine the level of the antinutritive factors at different stages of kochia growth,
3. to compare the amounts of antinutritive constituents in three varieties of kochia,
4. to determine the influence of location/season and stem colour on the level of the antinutritive factors.

2. MATERIALS AND METHODS

2.1 Samples

Kochia was grown on a saline site in Swift Current, Saskatchewan in 1985. Representative samples (six replicates) at all stages of maturity, were harvested on the following dates:

Prebud -	July 5, 1985
Bud to Early Flower -	July 15, 1985
Flower -	July 31, 1985
Early Fruit -	August 16, 1985
Mature Seed -	October 1, 1985

In addition to these samples, kochia grown from Texas seeds in Swift Current and plants of a Saskatchewan variety from wild stands in Radville, Saskatchewan, were harvested in 1985.

In 1986, kochia plants were harvested from a wild stand on the University of Saskatchewan (U of S) site. Kochia (six replicates) was harvested at five stages of maturity on the following dates:

Prebud -	July 4, 1986
Bud to Early Flower -	July 16, 1986
Early Flower -	August 6, 1986
Flower -	August 18, 1986
Early Fruit -	September 2, 1986

Samples of good (lavish, green vegetation, tall plants) and poor (yellow, short plants) quality were harvested from the U of S site.

Individual plants of kochia, characterized by red stems and red flowers or green stems and yellow flowers, were harvested in 1985 from the U of S site.

Samples were air dried, ground to pass through a 1 mm screen of the Wiley Mill and the composite samples of each of the stages of maturity were prepared for analysis.

For comparison purposes samples from New Mexico were included. Samples of low and high alkaloid content were obtained from Dr. Stan Smith from the University of New Mexico. These samples had been used in rat and sheep feeding trials in New Mexico.

2.2 Analysis

Samples were subjected to the following determinations:

- Total phenols were determined according to Swain and Hillis (1959). The procedure is based on the colour development between a Folin-Denis reagent and phenols present in a 80% ethanol extract of plant material. The concentration of total phenols is calculated from the standard of catechin solution in 80% ethanol:
- Phenolic acids were determined according to the methodology developed by Dabrowski and Sosulski (1984). Phenolic acids were fractionated into free, esterified and bound fractions. Free phenolic acids were extracted from kochia with THF (tetrahydrofuran). The esterified phenolic acids were extracted into a mixture of methanol:acetone:water (7:7:7 v/v/v). The bound phenolic acids in residues, after THF and MeOH:Acetone:H₂O extractions, were hydrolysed with 4N sodium hydroxide, followed by extraction of released phenolic acids in diethyl ether:ethyl acetate:tetrahydrofuran mixture. After evaporation to dryness, the residue was derivatized with BSA (Pierce Chemicals, Co.), and analysed on a capillary column of a Hewlett Packard GLC.

The GLC procedure was developed at SRC. The oven was programmed from 130°C to 312°C, at rate of 5°C/min. for 10 min., 2°C/min for 13 min., 4°C/min for 7 min. and 7°C/min. to 312°C. The flow rate of carrier gas, (helium) was 60 ml/min. The injection port was kept at 250°C and the flame ionization detector was at 300°C.

The p-hydroxyphenol acetic acid was used as an internal standard.

- Flavonols were determined according to the Swain and Hillis (1959), procedure using the vanillin reagent to develop the colour between flavonols and vanillin. The intensity of colour was measured on a Pye Unicam Spectrophotometer SP8-500.
- Saponins were analysed using the hemagglutination procedure of Jones and Elliott (1969). Hemolytic activity was defined as the lowest concentration of plant extract which will cause complete hemolysis of sheep red blood cells.
- Fibre was determined as the neutral detergent fibre, acid detergent fibre and acid detergent lignin, following the procedure described by Goering and Van Soest (1970).

3. RESULTS AND DISCUSSION

3.1 Poor Quality Kochia

3.1.1 Fibre

Total cell wall fibre in kochia, determined as neutral detergent fibre (NDF), constituted 40 to 60% of the plants' dry matter (DM) (Table 1). Young plants, at bud stage, contained 42.3% of NDF which was made up of 36.5% hemicellulose and 52.5% cellulose. The proportion of lignin in NDF was low.

More mature plants contained even higher proportion of cellulose as compared to hemicellulose in the total NDF (Table 1). The concentration of lignin increased significantly from 11 to 14.8% of the total NDF, as expected in more mature plants.

The NDF content will influence the digestibility of feed including the availability of vital constituents such as protein, fats and minerals. High concentration of lignin in NDF will suppress digestibility of kochia by ruminants.

3.1.2 Phenolic Acids

Phenolic acids (PA) in kochia at bud stage equaled 14 mg per g of plant material (Figure 1). The concentration of free phenolic acids (FPA) was the lowest while concentration of esterified phenolic acids (EPA) was the highest among three fractions. Bound phenolic acids (BPA) represented about 75% of the EPA.

At flower stage of kochia maturity the PA content reached the maximum level of 24 mg/g (Figure 1). However, at the later stages of growth the PA level declined and at the early fruit stage was almost equal to that at the bud stage.

Table 1

FIBRE CONTENT AND COMPOSITION OF KOCHIA FORAGE
HARVESTED AT DIFFERENT STAGES OF MATURITY.
(% DM)

Poor Quality Sample, 1986

Stage of Maturity	NDF	ADF	ADL	Hemi- cellulose	Cellulose
Bud	42.28	26.83	4.61	15.45	22.22
Early Flower	44.59	32.08	5.50	11.76	26.59

Figure 1.

PHENOLIC ACIDS IN KOCHIA

Poor Quality Sample, Saskatchewan, 1986

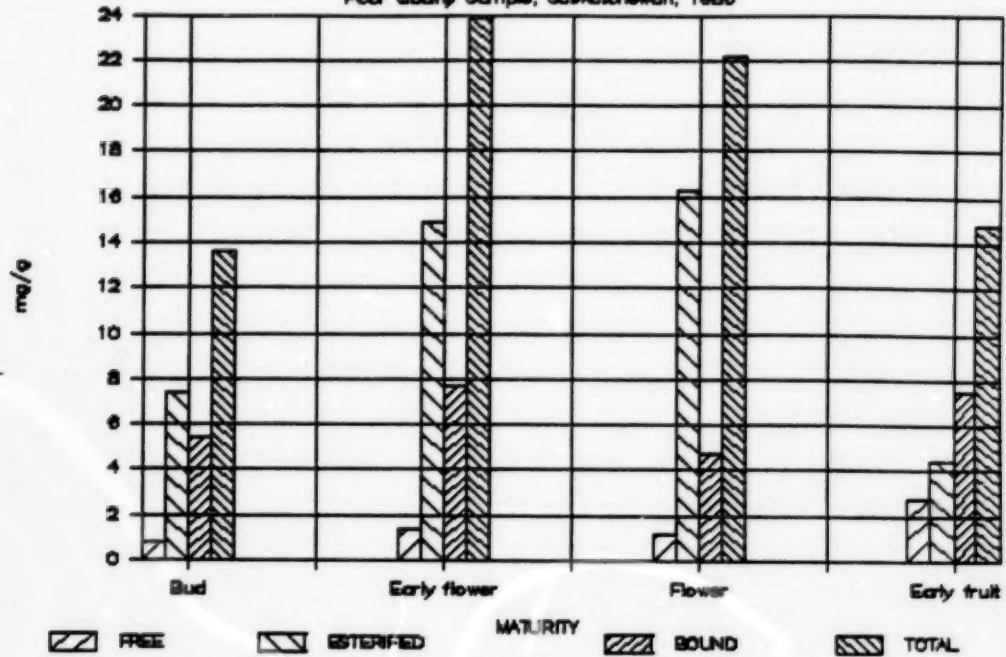
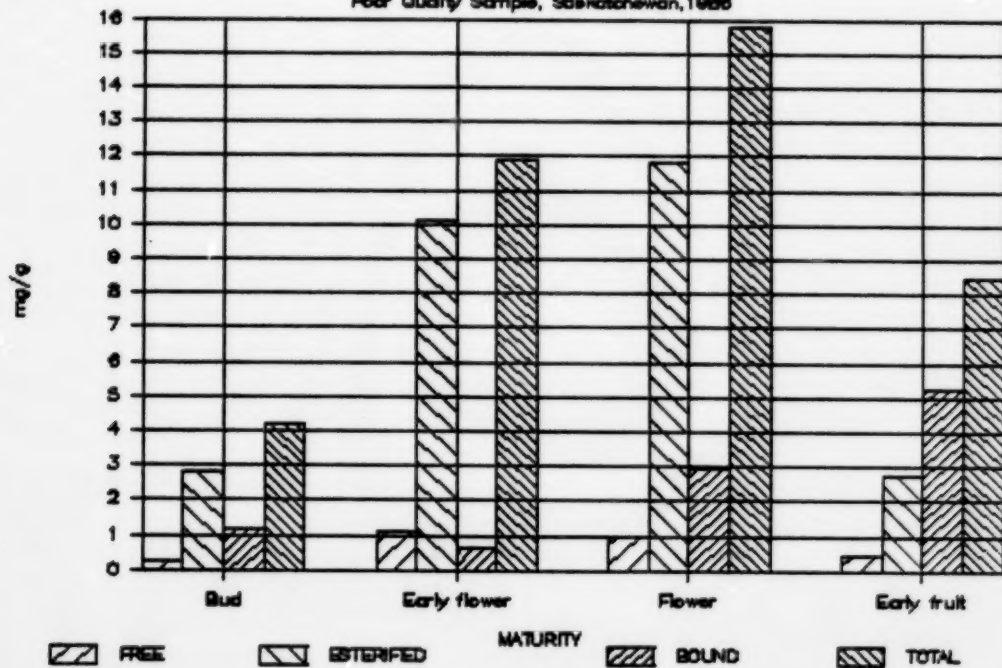


Figure 2.

BENZOIC ACIDS IN KOCHIA

Poor Quality Sample, Saskatchewan, 1986



The concentration of FPA fraction changed only marginally during plant growth (Figure 1). The EPA fraction was subjected to the greatest changes. During development, from bud to flower stage, the EPA almost doubled in concentration. But by the early fruit stage, EPA declined to a level below that of the plants' at the bud stage.

The concentration of PA in BPA fraction increased 40% over the level at bud stage (Figure 1).

The major PA in the FPA fraction were gentisic, protocatechuic and p-coumaric (Table 2). Protocatechuic, ferulic and caffeic were the most important in the EPA fraction (Table 3). In this fraction, gentisic and ferulic acids were especially high at early flower stage, but their level decreased with plant maturity.

The BPA fraction contained mainly ferulic, p-coumaric and protocatechuic acids (Table 4). Ferulic acid was the highest at the early flower stage.

The PA can be separated into two classes based on the number of carbon atoms in their molecule. The benzoic acids group (C_6-C_1) includes gentisic, protocatechuic and syringic acids, while the cinnamic acids group (C_6-C_3) includes p-coumaric, caffeic, isoferulic, ferulic, sinapic and chlorogenic acids.

Benzoic acids constituted 31% of the total phenolic acids at the bud stage and 51% at the early flower stage (Figure 2, Table A-1). The highest level of benzoic acids, though, was observed at the flower stage, 71% of the total PA. Cinnamic acids declined from 69% at the bud stage to 29% at the flower stage (Figure 3, Table A-1). At the early fruit stage this group of PA represented 43% of the total PA.

TABLE 2

FREE PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN

IN 1986. POOR QUALITY SAMPLE (MG/G)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
July 16	Bud	0.090	0.050	0.100	0.090	0.070	—	0.080	0.060	0.250	0.790
August 5	Early Flower	0.530	0.450	0.130	—	—	—	0.060	0.040	0.130	1.340
August 18	Flower	0.440	0.420	0.150	—	—	—	0.030	0.060	0.060	1.160
September 2	Early Fruit	0.213	0.190	0.050	1.700	0.130	0.210	0.120	0.100	0.070	2.783

TABLE 3

ESTERIFIED PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN

IN 1986. POOR QUALITY SAMPLE (MG/G)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
July 16	Bud	0.290	2.012	0.490	0.310	2.380	1.890	--	--	--	7.372
August 5	Early Flower	3.200	6.310	0.630	0.410	0.470	3.850	--	--	--	14.870
August 18	Flower	0.820	10.580	0.460	0.130	0.450	1.500	2.030	0.310	--	16.280
September 2	Early Fruit	0.670	0.120	1.940	0.260	0.350	0.600	0.240	0.300	--	4.480

TABLE 4

BOUND PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN

IN 1986. POOR QUALITY SAMPLE (MG/G)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
July 16	Bud	0.610	0.580	—	0.600	0.520	2.980	0.080	0.060	—	5.430
August 5	Early Flower	0.430	0.210	—	1.080	—	5.970	—	—	—	7.690
August 18	Flower	0.440	0.250	2.240	0.180	0.600	0.720	0.100	0.190	—	4.720
September 2	Early Fruit	0.160	4.970	0.150	0.590	0.160	1.420	0.080	—	—	7.530

The distributions of benzoic and cinnamic acids in free, esterified and bound PA fractions are presented on Figure 2 and 3. Benzoic acids consisted the majority of PA of free, esterified and bound fractions at early flower, flower, and early fruit stages, while cinnamic acids were the major PA at the bud stage.

3.1.3 Other Constituents

The concentration of total phenols almost doubled with maturity of kochia from 13.6 mg/g in young plants to 23.9 mg/g of flowering kochia (Table 5). Thereafter total phenols declined to 14.8 mg/g at the early fruit stage.

Flavonols increased from 7.3 mg/g of plant material at the bud stage to 15.8 mg/g at the flower stage (Table 5). At fruit stage there was only a small decline in flavonols content.

Concentration of tannins in the forage was almost unchanged during plant growth, from bud to flower (Table 5). At early fruit stage, however, there was a substantial increase in tannins in kochia.

Saponins also increased at the fruit stage (Table 5). The increase, however, was less than 10% of the value at the flower stage.

The relationships among constituents determined in kochia are presented on Figure 4. It appears that total PA, tannins and saponins increased with plant maturity. The PA and flavonols peaked at flower stage. Tannins increased in early fruit stage. Total phenols, though, decreased with plant maturity.

Figure 3.

CINNAMIC ACIDS IN KOCHIA

Poor Quality Sample, Saskatchewan 1988.

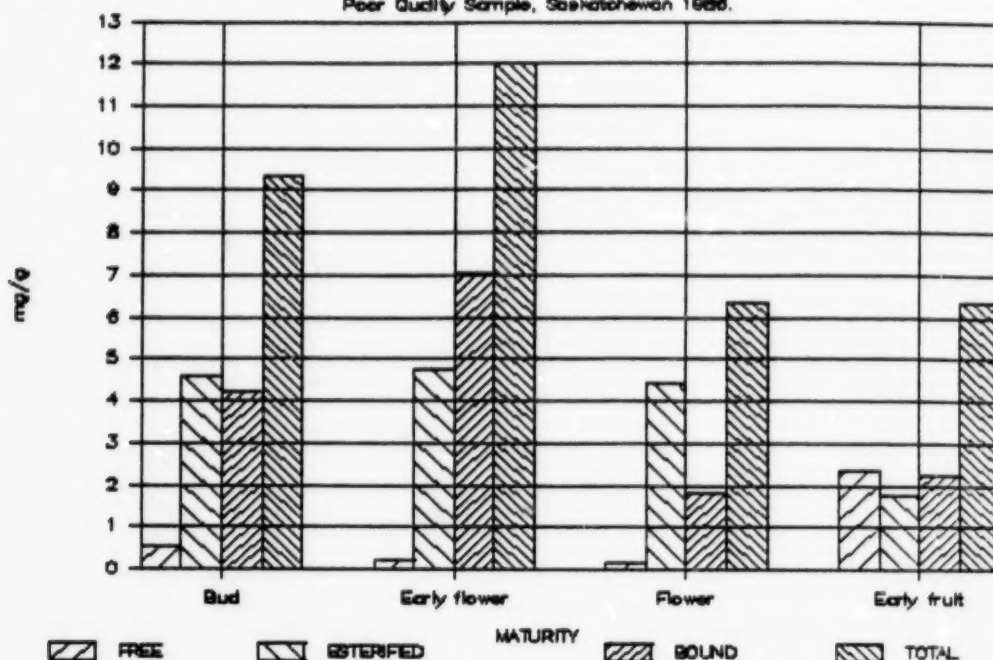


Figure 4.

CONSTITUENTS OF KOCHIA FORAGE

Poor Quality Sample, harvested 1988

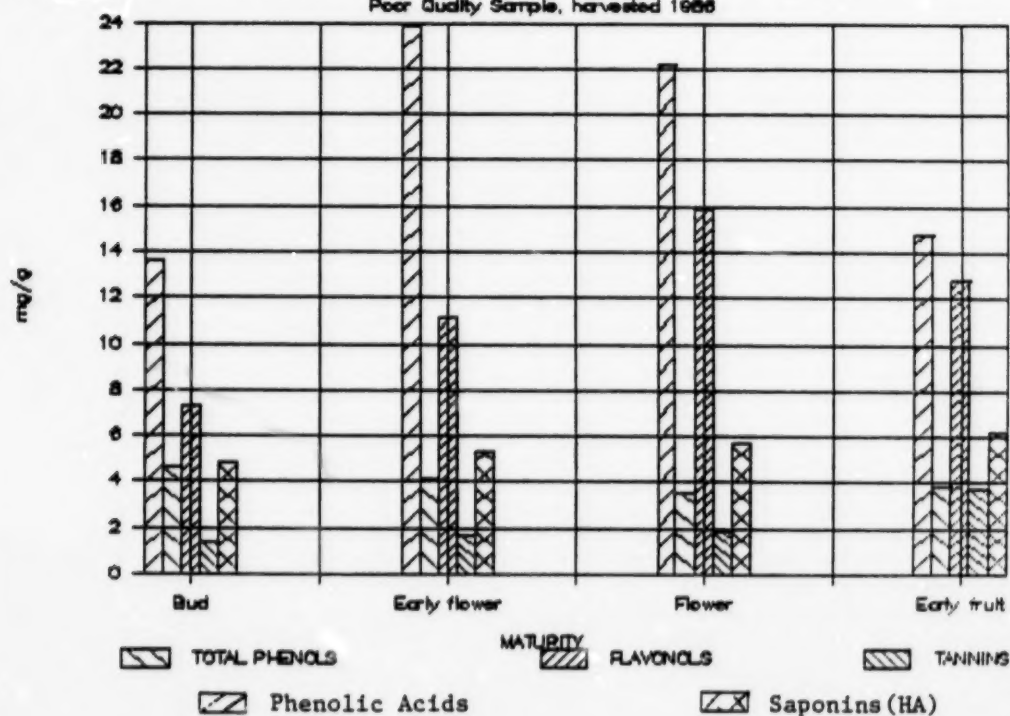


TABLE 5
EFFECT OF STAGE OF MATURITY OF POOR QUALITY
Kochia on constituents of forage
harvested in 1986

STAGE OF MATURITY	TOTAL PHENOLIC ACIDS mg/G	TOTAL PHENOLS mg/G	FLAVONOLS mg/G	TANNINS mg/G	SAPONINS HA/g
Bud	13.59	4.68	7.32	1.35	4.87
Early Flower	23.90	4.17	11.11	1.70	5.37
Flower	22.16	3.58	15.85	1.90	5.73
Early Fruit	14.79	3.86	12.79	3.73	6.25

3.2 Good Quality Kochia

3.2.1 Fibre

Total NDF content was in the range of 37-45% of DM, with the highest value occurring at the flower stage (Table 6). These values were 3-5% lower than corresponding values for poor quality kochia, harvested at the same stage of maturity and from the same site (Table 1).

Good quality kochia contained less hemicellulose, a comparable amount of cellulose and less lignin, than poor quality samples (Tables 1 and 6).

3.2.2 Phenolic Acids

The content of PA in kochia was influenced by forage quality. Good quality sample contained less of PA as compared to poor quality forage (Figure 1 and 5). Total PA were in the range of 10-16.2 mg/g, which was 29-34% less than values for poor quality samples. The highest concentration of PA was at the early fruit stage.

The EPA was the predominant fraction of PA present in kochia at early bud stage (Figure 5). At bud, early flower and flower stages of growth the majority of PA were accumulated in the BPA fraction.

Gentisic and protocatechuic acids were predominant in FPA fraction of good quality kochia (Table 7). Isoferulic, ferulic, caffeic and sinapic acids were present in small concentrations only. Gentisic and protocatechuic acids were also the major PA in esterified fraction (Table 8). However, the concentrations of isoferulic, ferulic, caffeic and sinapic acids in EPA were several times higher than in the FPA fraction.

Table 6

FIBRE CONTENT AND COMPOSITION OF KOCHIA FORAGE
HARVESTED AT DIFFERENT STAGES OF MATURITY.
(% DM)

Good Quality Sample, 1986

Stage of Maturity	NDF	ADF	ADL	Hemi- cellulose	Cellulose
Early bud	36.98	28.08	4.26	9.03	23.80
Bud	37.99	29.03	4.52	8.96	24.51
Early Flower	39.44	29.65	5.69	9.80	23.96
Flower	45.25	31.14	5.77	14.11	25.36
Early Fruit	42.70	30.43	6.05	12.27	24.38

Figure 5.

PHENOLIC ACIDS IN KOCHIA.

Good Quality Sample, Saskatchewan, 1986

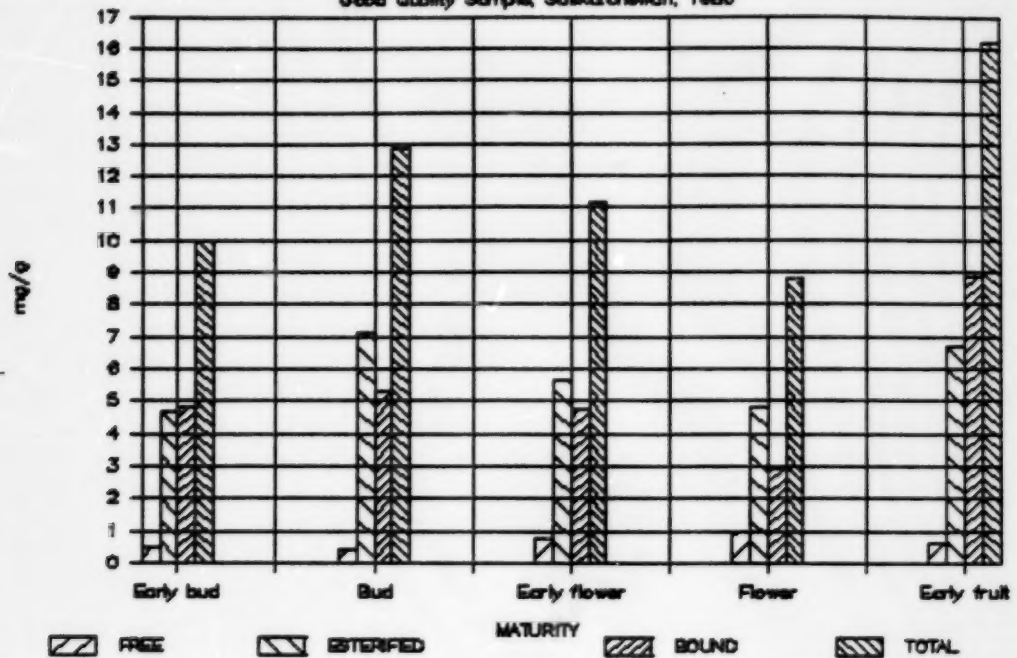


Figure 6.

BENZOIC ACIDS IN KOCHIA

Good Quality Sample, Saskatchewan, 1986

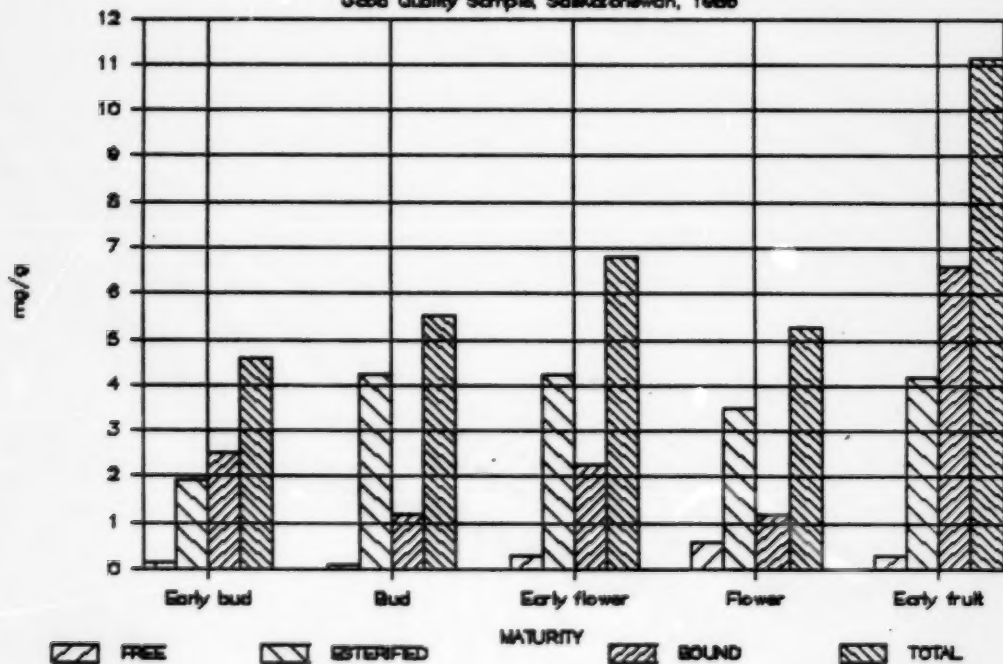


TABLE 7

FREE PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN

IN 1986. GOOD QUALITY SAMPLE (MG/G)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
July 4	Early Bud	0.080	0.050	—	0.020	—	—	0.040	0.040	0.230	0.460
July 16	Bud	0.040	0.080	—	—	—	—	0.030	0.050	0.210	0.410
August 6	Early Flower	0.190	0.030	0.070	0.034	0.011	0.050	0.080	0.060	0.250	0.775
August 18	Flower	0.197	0.270	0.100	0.165	0.090	0.028	0.030	—	0.100	0.980
September 2	Early Fruit	0.230	0.050	0.040	0.166	—	—	—	0.068	0.060	0.614

TABLE 8

ESTERIFIED PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN IN

1986. GOOD QUALITY SAMPLE (MG/G)

Date of Harvesting	Stage of Maturity	Gentic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isofenu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
July 4	Early Bud	1.090	0.160	0.670	0.140	0.190	1.380	0.580	0.490	0.003	4.703
July 16	Bud	1.490	2.740	—	—	0.750	1.130	0.468	0.560	—	7.138
August 6	Early Flower	1.000	2.490	0.750	0.140	0.530	0.130	0.310	0.280	—	5.630
August 18	Flower	1.190	1.570	0.760	0.220	0.450	0.330	0.130	0.180	—	4.830
September 2	Early Fruit	1.330	1.600	1.270	0.70	0.210	0.910	0.570	0.130	—	6.720

The BPA contained mainly gentisic, protocatechuic and syringic acids. Ferulic acid occurred at high concentration only at early growth stages (Table 9).

Good quality kochia contained less ferulic, isoferulic and caffeic acids in both esterified and bound phenolic acid fractions as compared to poor quality sample (Tables 2, 4, 8, and 9).

Most of phenolic acids in good quality kochia, harvested in 1986, were of the benzoic acids group (C_6-C_1) (Figure 6). However, their concentrations were lower than in poor quality samples (Figure 2).

Benzoic acids in good quality kochia constituted for 45.8-68.7% of the total PA depending on growth stage (Figure 6, Table A-2). At early flower stage their concentration was the greatest among samples from early bud to flower stage of growth. A sudden increase in the benzoic acids was detected at early fruit stage. Poor quality sample had a maximum benzoic acid concentration at the flower stage (Figure 2).

Cinnamic acids occurred in much higher concentration in poor quality kochia (Figure 3) than in good quality one (Figure 7, Table A-2). These PA were present mainly in early bud, bud and early flower stages of plant growth of good, as well as, poor quality forages. Cinnamic acids accumulated mainly in the BPA fraction.

3.2.3 Other Constituents

A similar level of total phenols was determined in good quality and in poor quality samples of kochia (Figure 8). Total phenols remain almost unchanged during kochia growth.

Flavonols low in early bud or bud stage samples though, increased 4 to 7 times by the early flower and flower stages, resp. (Table 10).

TABLE 9

BOUND PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN

IN 1986. GOOD QUALITY SAMPLE (MG/G)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
July 4	Early Bud	1.241	1.290	—	0.430	—	1.480	0.390	—	—	4.831
July 16	Bud	0.610	0.580	—	0.600	0.520	2.980	—	—	—	5,290
August 6	Early Flower	0.710	0.610	0.950	2.210	—	—	0.170	0.100	—	4.750
August 18	Flower	0.790	0.560	1.430	—	—	—	0.120	0.058	—	2.958
September 2	Early Seed	0.160	4.970	1.500	0.590	0.160	1.420	0.080	—	—	8.890

Figure 7.

CINNAMIC ACIDS IN KOCHIA

Good Quality Sample, Saskatchewan, 1986

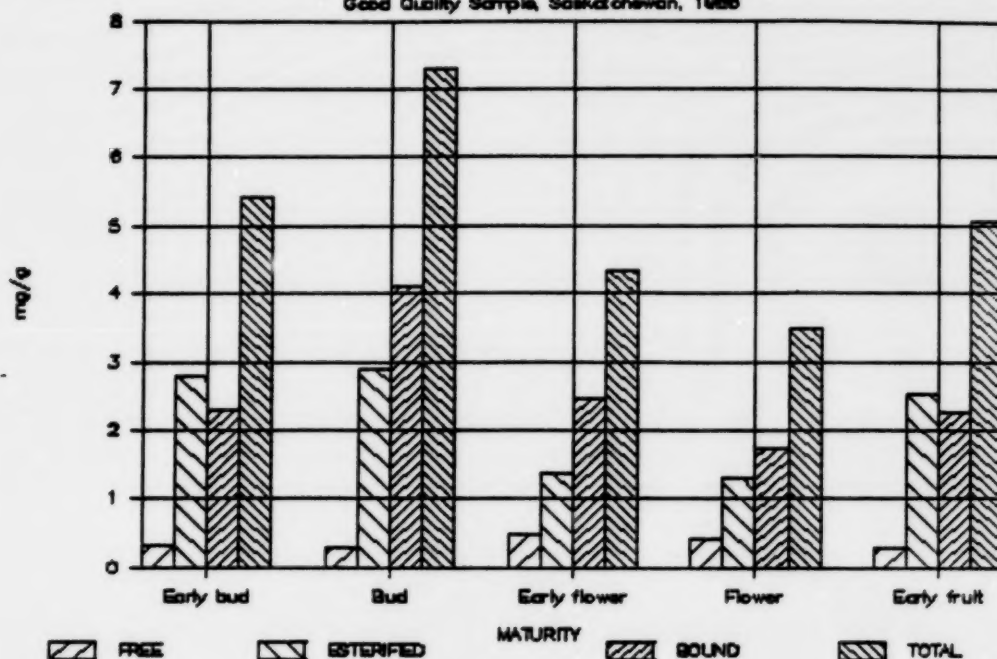
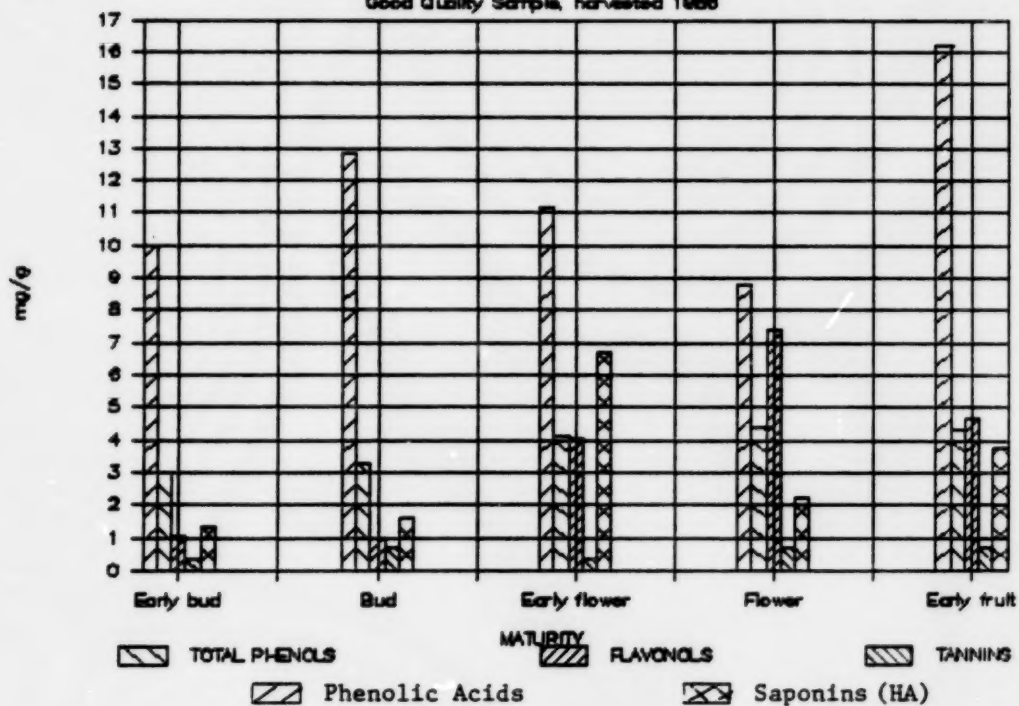


Figure 8.

CONSTITUENTS OF KOCHIA FORAGE

Good Quality Sample, harvested 1986



Peak concentration of flavonols was obtained at flower stage and there was their decrease at early fruit stage.

Tannins were present in a very small concentration (Table 10). The highest value, at early fruit stage, was much lower than the lowest value in poor quality sample, i.e. 0.7 as compared to 1.3 mg/g, resp. (Tables 5 and 10). Low concentration of tannin in good quality kochia could represent a more palatable forage.

Saponins in good quality kochia were present in approximately one-half of the amount determined in poor quality sample (Tables 5 and 10). The highest concentration of saponins and the highest hemagglutination activity were determined at early flower stage.

Good quality kochia contained less of all of the antinutritive constituents analysed, as compared to poor quality one (Tables 5 and 10).

Total phenols and tannins increased with maturity of forage. There was a peak level followed by a decline in PA content (bud stage), flavonols content (early flower stage) and saponin activity (also at early flower stage).

3.3 Good Quality Kochia

Good quality kochia harvested on a saline site at Swift Current in 1985 was included in the present discussion for comparison purpose.

3.3.1 Fibre

Kochia harvested in 1985 contained less of total NDF than one harvested in 1986 (Tables 6 and 11). The fibre content increased with maturity of sample.

TABLE 10
EFFECT OF STAGE OF MATURITY OF GOOD QUALITY
KOCHIA ON CONSTITUENTS OF FORAGE
HARVESTED IN 1986

STAGE OF MATURITY	TOTAL PHENOLIC ACIDS mg/G	TOTAL PHENOLS mg/G	FLAVONOLS mg/G	TANNINS mg/G	SAPONINS HA/g
Early Bud	9.99	3.02	1.03	0.35	1.31
Bud	12.84	3.27	0.99	0.68	1.62
Early Flower	11.16	4.14	4.07	0.38	6.70
Flower	8.77	4.40	7.39	0.69	2.22
Early Fruit	16.22	4.30	4.69	0.73	3.76

Table 11

FIBRE CONTENT AND COMPOSITION OF KOCHIA FORAGE
HARVESTED AT DIFFERENT STAGES OF MATURITY.
(% DM)

Good Quality Sample, 1985

Stage of Maturity	NDF	ADF	ADL	Hemi- cellulose	Cellulose
Early bud	32.3	18.0	4.0	14.3	14.0
Bud	35.0	19.7	3.6	15.3	16.1
Flower	40.3	20.9	4.5	19.4	16.4
Early Fruit	41.2	22.1	5.5	15.1	16.6
Mature Seed	54.2	27.6	8.8	26.6	18.8

Hemicellulose was the major component of kochia at various stages of maturity and constituted from 44.3 to 49% of the total NDF (Table 11). Cellulose was in the range of 14-18.8% of DM or 34.6-46% of the total NDF. Kochia from the 1985 growing season contained more hemicellulose as compared to that from 1986.

It appears that fibre content and concentration are influenced by environmental conditions, as well as soil quality.

3.3.2 Phenolic Acids

Kochia grown on saline soil had increased levels of PA as compared to that from the wild stand at the U of S site.

Maximum PA in FPA fraction was determined at bud stage and, thereafter, the level of phenolic acids decreased (Figure 9). During the subsequent stages of growth the PA level was unchanged. The EPA declined slowly with maturity, while BPA increased at bud stage only to decline sharply at flower and early fruit stages. The PA content in kochia at mature fruit stage, was very low, mainly due to the loss of leaves.

Major PA in FPA fraction were protocatechuic and chlorogenic acids (Table 12). The EPA fraction though, contained mainly gentisic and protocatechuic acids with p-coumaric, isoferulic, ferulic and caffeic acids being present in smaller concentrations (Table 13). The highest level of isoferulic acid was present in kochia at early fruit stage, while ferulic acid was present in a similar range at all stages of maturity.

The BPA fraction was rich in gentisic, protocatechuic, syringic and p-coumaric acids (Table 14).

Figure 9. PHENOLIC ACIDS IN KOCHIA mg/g

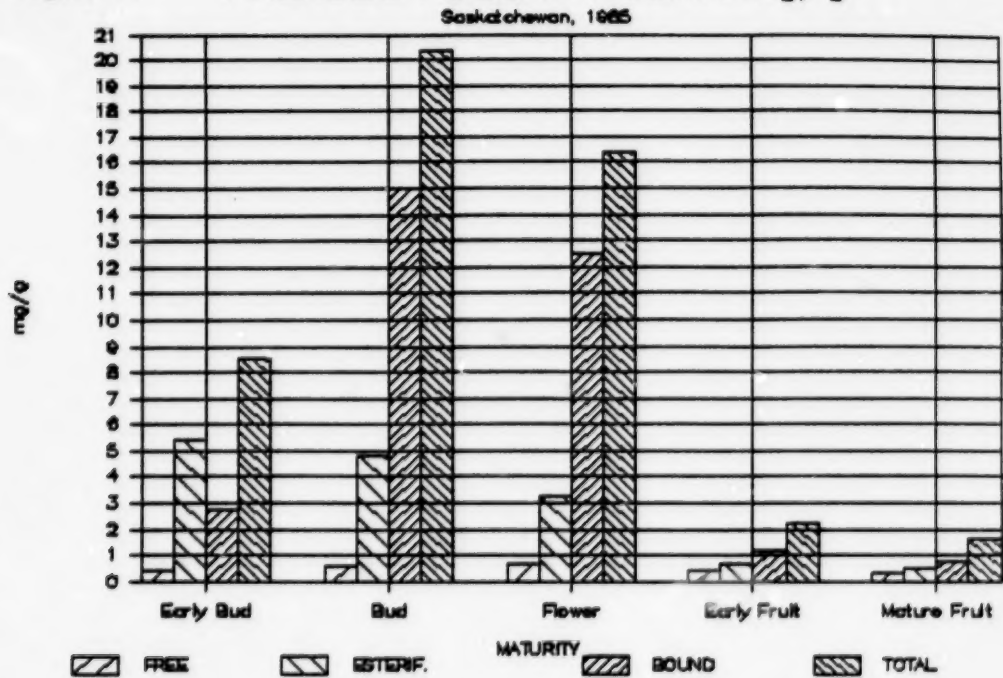


Figure 10. BENZOIC ACIDS IN KOCHIA

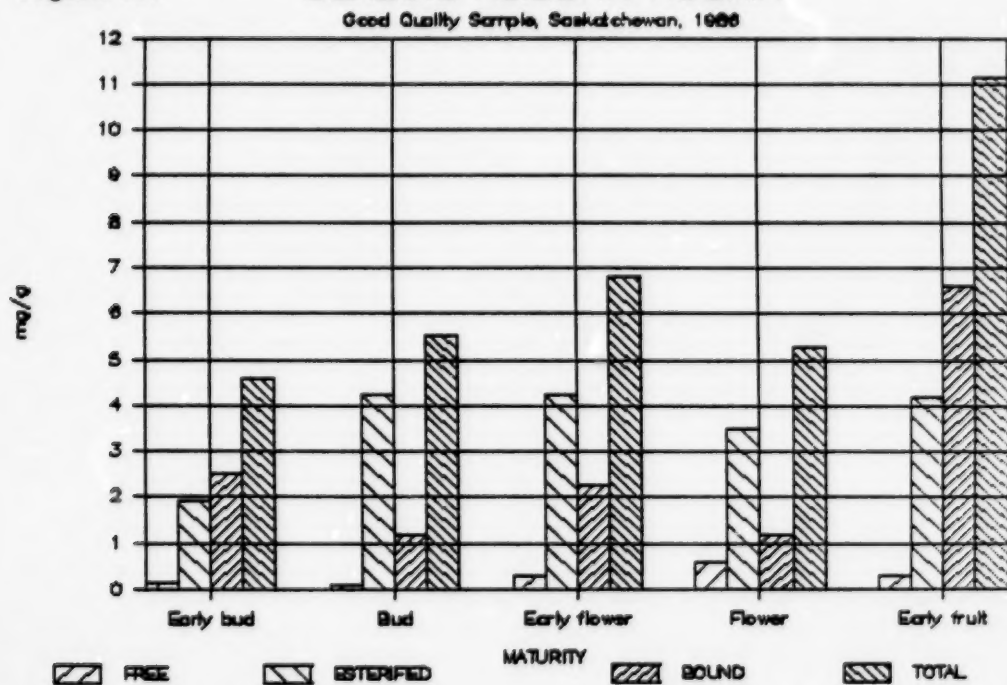


Table 12

FREE PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN
IN 1985. (mg/g)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Proto Catechuic Acid	Syringic Acid	Para Coumaric Acid	Isoferulic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chlorogenic Acid	Total
July 5	Early bud	0.036	0.161	0.013	-	0.011	-	0.015	0.015	0.169	0.421
July 15	Bud	0.039	0.229	0.065	-	0.014	-	0.010	0.019	0.199	0.575
July 31	Flower	0.037	0.204	0.082	-	0.019	-	0.015	0.074	0.219	0.650
August 6	Early Fruit	0.091	0.118	0.054	-	-	-	-	0.170	0.098	0.433
October 1	Mature Fruit	0.031	0.223	0.052	-	-	-	-	-	0.030	0.336

Table 13
ESTERIFIED PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN
IN 1985. (mg/g)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Proto Catechuic Acid	Syringic Acid	Para Coumaric Acid	Isoferulic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chlorogenic Acid	Total
July 5	Early bud	2.679	2.028	-	0.089	0.020	0.077	0.492	0.036	-	5.421
July 15	Bud	1.798	2.465	-	0.611	0.244	0.118	0.097	0.064	-	4.847
July 31	Flower	1.749	0.181	0.403	0.110	0.102	0.085	0.176	0.459	-	3.265
August 6	Early Fruit	0.091	0.076	-	0.115	1.792	0.086	0.136	-	-	0.683
October 1	Mature Fruit	0.049	0.042	-	0.121	-	0.061	0.169	0.075	-	0.518

As compared to kochia from 1986, the sample harvested in 1985 contained similar levels of PA in FPA fraction (Tables 7 and 12). Both EPA fractions were characterized by a maximum PA content at bud stage, however, kochia from 1985 accumulated less ferulic acid as compared to the 1986 sample (Tables 8 and 13). The BPA fraction, though, was much greater in kochia from 1985 as compared to that in 1986 (Tables 9 and 14).

Benzoic acids accounted for 80-85% of the total PA at early bud and bud stage (Figure 12, Table A-3). At later stages of growth their concentration declined to 49.8-29.7% of the total PA for early fruit and mature fruit, resp.

Cinnamic acid concentrations were low in young plants but increased with maturity up to 72% of the total PA (Fig 12, Table A-3).

As compared to good quality kochia from 1986 harvest this sample contained much more of the benzoic acids (Tables A-2 and A-3). Cinnamic acids were present in lower concentration. Poor quality kochia from 1986 contained only one-half of the level of benzoic acids determined in kochia from 1985 season (Tables A-1 and A-3).

Also the distribution of the individual PA varied with quality/-growing season and/or location. Kochia from 1985 had the lowest levels of ferulic, isoferulic and caffeic acids (Tables 12, 13 and 14).

3.3.3 Other Constituents

Kochia from the saline site contained much higher contents of the total phenols, as compared to samples of poor and good quality kochia from 1986 harvest (Tables 5, 10 and 15). The value determined for total phenols, at early bud stage, was twice as high as in 1986's samples

Table 14
BOUND PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN
IN 1985. (mg/g)

Date of Harvesting	Stage of Maturity	Gentisic Acid	Proto Catechuic Acid	Syringic Acid	Para Coumaric Acid	Isoferulic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chlorogenic Acid	Total
July 5	Early bud	-	0.489	1.934	-	0.073	0.161	0.058	0.020	-	2.735
July 15	Bud	2.679	6.355	2.761	2.955	-	0.229	-	-	-	14.979
July 31	Flower	2.908	1.010	1.614	0.651	1.672	0.464	2.093	2.100	-	12.512
August 6	Early Fruit	0.148	0.100	-	0.338	-	0.486	-	0.091	-	1.163
October 1	Mature Fruit	0.011	0.045	-	0.014	-	0.729	-	0.015	-	0.814

Figure 11.

CINNAMIC ACIDS IN KOCHIA

Saskatchewan, 1986

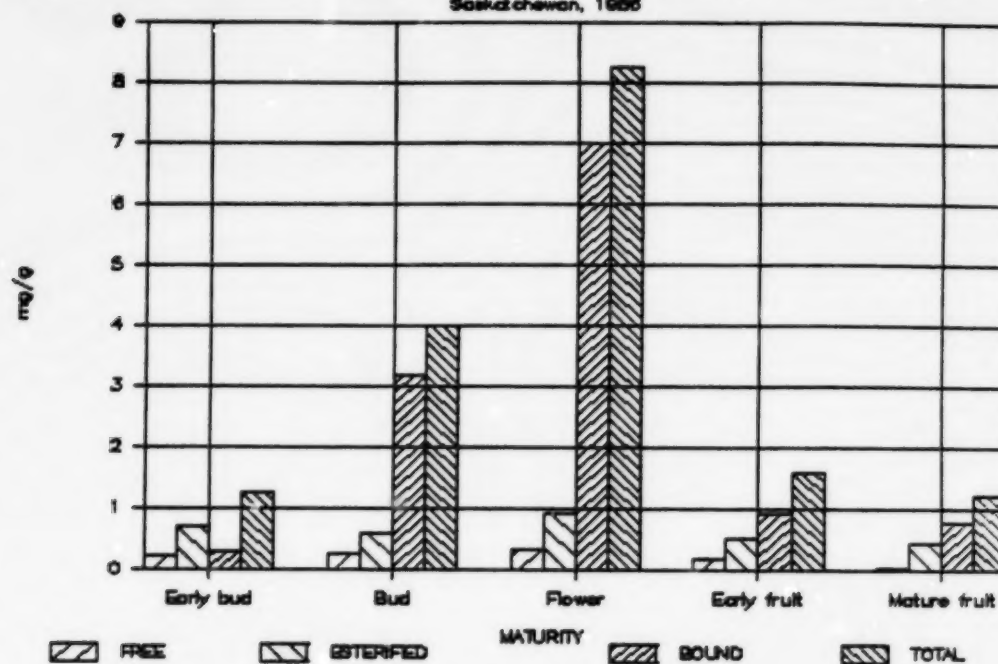
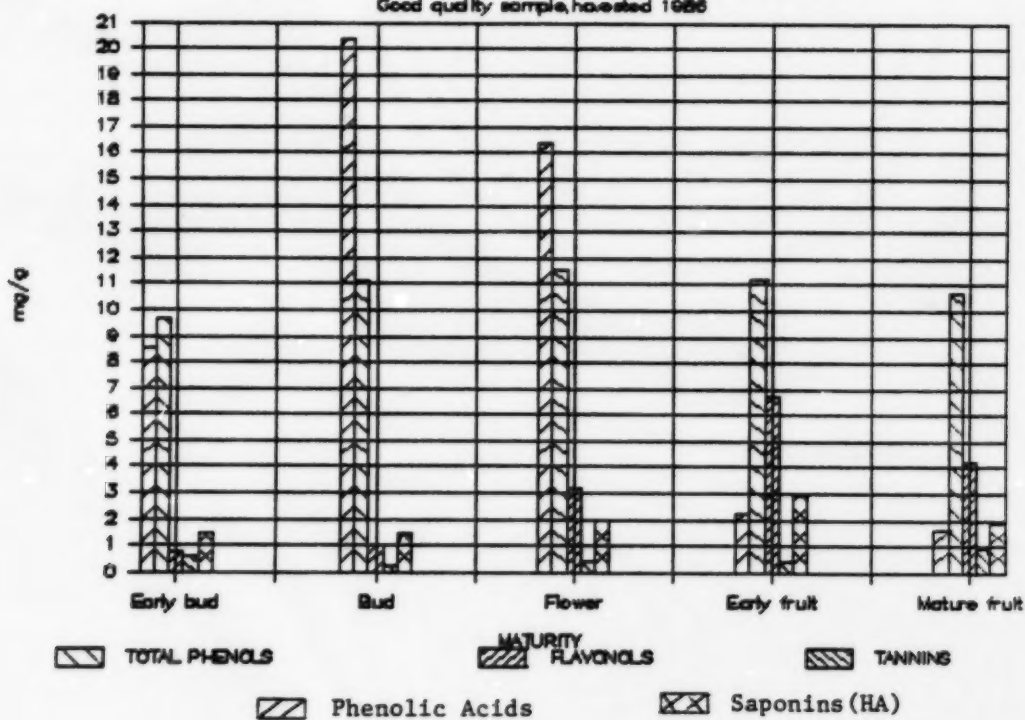


Figure 12.

CONSTITUENTS OF KOCHIA FORAGE

Good quality sample, harvested 1986



(Table 15). A further increase was observed at bud stage and this value remained stable during the next two stages of growth.

Flavonols, though, were present in the amounts similar to those in kochia of good quality, harvested in 1986 (Tables 10 and 15). These values were 10 to 3 times lower than in poor quality kochia (Tables 5 and 15). Flavonols increased with plant maturity and maximum values were found at early fruit stage.

Comparable values of tannins and saponins were determined in both samples of good quality kochia from 1985 and 1986 (Table 15).

It appears that the antinutritive constituents in kochia are influenced by soil quality, both lack of nutrients and salinity, and by location and environmental conditions.

Poor quality kochia contained up to 49 units of the total antinutrients (PA, total phenols, flavonols, tannins and saponins). Their content peaked at flower stage (Table 16).

Good quality kochia contained one-half of the total antinutrients determined in poor sample (Table 16). However, good quality kochia from saline soil (1985) contained more of the antinutritive compounds as compared to samples from 1986.

The highest content of the total antinutritive compounds was found in bud, early flower and flower stages of growth for kochia samples from three sites (Table 16). Therefore, it may be advisable to use kochia as feed for grazing animals at the early bud stage instead of the early flower or flower stages at which kochia is commonly used as forage.

TABLE 15
EFFECT OF STAGE OF MATURITY OF GOOD QUALITY
KOCHIA ON ANTINUTRITIVE CONSTITUENTS OF FORAGE
HARVESTED IN 1985

Stage of Maturity	Total Phenolic Acids mg/G	Total Phenols mg/G	Flavonols mg/G	Tannins mg/G	Saponins HA/g
Early Bud	8.58	9.70	0.77	0.61	1.46
Bud	20.40	11.13	1.04	0.24	1.43
Flower	16.43	11.60	3.18	0.41	2.01
Early Fruit	2.28	11.20	6.75	0.40	2.93
Mature Fruit	1.67	10.70	4.25	0.93	1.88

TABLE 16
TOTAL OF ANTINUTRITIVE CONSTITUENTS IN KOCHIA

Stage of Maturity	1986 Poor Quality	1986 Good Quality	1985 Good Quality
Early Bud	--	15.70	21.12
Bud	31.81	19.40	34.41
Early Flower	46.25	26.55	--
Flower	49.22	23.47	23.59
Early Seed	41.42	29.70	19.43
Mature Seed	--	--	19.43

3.4 Native, Texas and New Mexico Kochia

Comparison of kochia grown in Saskatchewan, from seeds obtained from native and Texas origin plants, is presented in Table 17. All samples contained relatively low amounts of PA. Native and Texas origin kochia were comparable, while New Mexico kochia contained a slightly lower level of PA. Also, total phenols were lower in New Mexico kochia as compared to samples grown in Saskatchewan. Flavonols content though, was the lowest in the Texas variety and the highest in New Mexico material with native kochia being intermediate. The New Mexico strain had the highest tannin and saponin contents.

The total of all antinutritive compounds was the highest in the New Mexico material (38.49) and the lowest in Texas-origin material grown in Saskatchewan (28.53). Native kochia was intermediate, with value of 33.73. Although the "total of antinutritive compounds" is not a widely recognized unit of measurement but the value is useful in evaluation of the quality of the forage. Papers from the New Mexico reported that their kochia, especially at the flower stage, are toxic to grazing animals and this information may have some relevance to presented data. In addition, oxalate content, and alkaloid content, not measured in this report, may be factors in reported instances of toxicity in kochia.

3.5 Other samples of kochia

Kochia with red leaves and red flowers was comparable in PA content to green stem and yellow flower plant (Table 18). However, red stem kochia contained more of total phenols, flavonols, tannins and saponins as compared to green stem kochia grown at the same location.

The majority of the phenolic components of kochia were concentrated in the leaf fraction (Table 19). The stem fraction contained less of all antinutritive compounds.

TABLE 17
CONTENT OF ANTINUTRITIVE CONSTITUENTS IN KOCHIA GROWN IN SASKATCHEWAN FROM
THE NATIVE AND TEXAS ORIGIN SEEDS AND IN KOCHIA SAMPLE FROM
NEW MEXICO (AVERAGE VALUES FOR VARIETY)

Variety of Kochia	Total PA mg/g	Total Phenols mg/g	Flavonols mg/g	Tannins mg/g	Saponins HA/g
Native	8.70	4.30	12.70	2.70	5.33
Texas	8.83	4.05	7.40	3.10	5.15
New Mexico	7.55	3.35	17.0	4.50	6.10

TABLE 18
CONTENT OF ANTINUTRITIVE CONSTITUENTS IN
RED AND GREEN STEM KOCHIA, SASKATCHEWAN, 1986

Kochia	Total PA mg/g	Total Phenols mg/g	Flavonols mg/g	Tannins mg/g	Saponins HA/g
Red	10.08	3.75	9.20	9.71	5.10
Green	10.07	2.20	7.18	7.01	3.21

TABLE 19
CONTENT OF ANTINUTRITIVE CONSTITUENTS IN KOCHIA FORAGE AND
LEAF AND STEM FRACTIONS (SAMPLE FROM 1983)

Sample	Total PA mg/g	Total Phenols mg/g	Flavonols mg/g	Tannins mg/g	Saponins HA/g
Forage	13.81	11.20	6.80	14.8	1.23
Leaf	15.39	16.20	14.20	18.0	1.23
Stem	13.38	6.50	3.50	9.2	1.37

4. CONCLUSIONS

1. Poor quality kochia with yellow leaves and short stature contained more NDF as well as all of four antinutritive components analyzed, as compared with good quality sample harvested from the same location.
2. There was a location/environmental difference among samples of good quality kochia grown in Saskatchewan. More of the antinutritive compounds accumulated in forage grown on saline than on regular soil.
3. The highest concentrations of the four antinutritive components were found in kochia from New Mexico. Texas-origin kochia contained less of these components than Saskatchewan-origin kochia.
4. Small differences in antinutritive constituent contents were determined between kochia with red stems and red flowers and those with green stems and yellow flowers.
5. Antinutritive compounds accumulated in the leaf fraction of kochia.
6. The relative amounts of phenolic acids and flavonols in kochia at the later stages of maturity suggest that both types of compounds could be significant contributors to the growth inhibiting (allelopathic) effects of kochia plant debris on the next year's plant growth.

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Table A-1

PERCENTAGE OF BENZOIC ACID AND CINNAMIC ACID IN TOTAL
PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN
IN 1986. POOR QUALITY SAMPLE

C6-C1
BENZOIC ACIDS

Date of Harvest	Stage of Maturity	Total P.A.	Percentage of Total P.A.
July 16	Bud	4.222	31.06
Aug. 5	Early Flower	11.890	49.75
Aug. 18	Flower	15.800	71.30
Sept. 2	Early Fruit	8.463	57.21

C6-C3
CINNAMIC ACIDS

Date of Harvest	Stage of Maturity	Total P.A.	Percentage of Total P.A.
July 16	Bud	9.370	68.94
Aug. 5	Early Flower	12.010	50.25
Aug. 18	Flower	6.360	28.70
Sept. 2	Early Fruit	6.330	42.79

Table A-2

PERCENTAGE OF BENZOIC ACID AND CINNAMIC ACID IN TOTAL
PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN
IN 1986. GOOD QUALITY SAMPLE

C6-C1
BENZOIC ACIDS

Date of Harvest	Stage of Maturity	Total P.A.	Percentage of Total P.A.
July 4	Early bud	4.581	45.84
July 16	Bud	5.540	43.15
Aug. 6	Early Flower	6.800	60.95
Aug. 16	Flower	5.287	60.30
Sept. 2	Early Fruit	11.150	68.72

C6-C3
CINNAMIC ACIDS

Date of Harvest	Stage of Maturity	Total P.A.	Percentage of Total P.A.
July 4	Early bud	5.413	54.16
July 16	Bud	7.298	56.85
Aug. 6	Early Flower	4.355	39.04
Aug. 16	Flower	3.481	39.70
Sept. 2	Early Fruit	5.074	31.27

Table A-3

PERCENTAGE OF BENZOIC ACID AND CINNAMIC ACID IN TOTAL
PHENOLIC ACIDS IN KOCHIA HARVESTED IN SASKATCHEWAN
IN 1985

C6-C1
BENZOIC ACIDS

Date of Harvest	Stage of Maturity	Total P.A.	Percentage of Total P.A.
July 5	Early bud	7.340	85.57
July 15	Bud	16.391	80.34
July 31	Flower	8.188	49.84
August 6	Early Fruit	0.678	29.75
October 1	Mature Fruit	0.453	27.16

C6-C3
CINNAMIC ACIDS

Date of Harvest	Stage of Maturity	Total P.A.	Percentage of Total P.A.
July 5	Early bud	1.237	14.42
July 15	Bud	4.010	19.66
July 31	Flower	8.239	50.16
August 6	Early Fruit	1.601	70.25
October 1	Mature Fruit	1.215	72.84

TABLE A - 4

PHENOLIC ACIDS IN KOCHIA SAMPLES HARVESTED IN NEW MEXICO (MG/G)

	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
<u>Free Phenolic Acids</u>										
Kochia A										
- low alkaloid cont.	0.100	0.240	0.040	0.180	0.020	-	0.010	0.010	0.050	0.650
Kochia B										
- high alkaloid cont.	0.110	0.500	0.020	0.070	0.030	0.020	0.100	0.070	0.040	0.960
Kochia C										
- rat feeding trial	0.113	0.140	0.260	0.140	0.080	-	0.120	0.130	0.046	1.026
<u>Esterified Phenolic Acids</u>										
Kochia A										
- low alkaloid cont.	0.900	1.360	0.280	0.570	0.290	0.280	0.290	0.190	-	4.160
Kochia B										
- high alkaloid cont.	1.240	2.120	0.210	0.200	0.250	0.170	0.040	0.150	-	4.380
Kochia C										
- rat feeding trial	0.970	0.560	0.700	0.120	0.250	0.140	0.200	0.130	-	3.070
<u>Bound Phenolic Acids</u>										
Kochia A										
- low alkaloid cont.	0.160	0.170	0.350	0.100	0.210	0.150	0.010	0.130	-	1.280
Kochia B										
- high alkaloid cont.	0.550	0.340	0.133	0.416	0.360	0.190	0.020	0.200	-	2.209
Kochia C										
- rate feeding trial	0.500	0.060	0.380	0.220	0.320	-	0.120	0.130	-	1.730

TABLE A - 5

THE INFLUENCE OF STEM COLOUR ON FREE ESTERIFIED
AND BOUND PHENOLIC ACIDS (mg/g)

Stem Colour	Gentisic Acid	Protoca- technic Acid	Syringic Acid	Para- coumaric Acid	Isoferu- lic Acid	Ferulic Acid	Caffeic Acid	Sinapic Acid	Chloro- genic Acid	Total
<u>Free Phenolic Acids</u>										
Green	0.430	0.110	0.020	0.020	0.010	0.080	0.010	0.020	0.210	0.910
Red	0.215	0.200	0.030	0.190	0.050	0.040	0.010	0.030	0.180	0.945
<u>Esterified Phenolic Acids</u>										
Green	1.390	0.980	0.230	0.280	0.290	0.280	0.240	0.120	0.030	3.840
Red	1.210	1.780	0.220	0.340	0.170	0.170	0.190	0.060	0.010	4.150
<u>Bound Phenolic Acids</u>										
Green	0.390	0.140	1.540	2.100	0.100	1.010	0.040	0.010	—	5.330
Red	0.770	0.180	0.970	2.080	0.150	1.300	0.080	0.040	—	5.570